VEGETABLE GERMPLASM COLLECTING

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Some Basic Considerations

Collecting germplasm is not an easy proposition. In the past the following problems were associated with collecting genetic diversity: time, money and danger. At present, germplasm collectors are faced with legal issues as well. The concepts of ownership, sovereignty and intellectual property rights will be affecting more and more access to germplasm. Therefore, increasingly, collecting will be done only very selectively.

Actual exploration and collecting is only one of the means to assemble a germplasm collection. Additional means are by donations and seed exchange with institutions or individuals holding a portion of the germplasm.

But, why assemble a germplasm collection? There are many reasons for the establishment of a germplasm collection. In many cases, a germplasm collection is assembled because there is a need for the germplasm in crop improvement programs. That is why initial germplasm collection is usually done by plant breeders. Plant breeders and other research workers require genetically diverse materials to be able to continuously develop improved types and varieties of crops that can face the challenge of an environment that is both evolving and becoming limiting. A genetically diverse genepool is the raw material from which the plant breeder will mold new varieties. The availability of specific genes in the collection determines the success of the plant breeder in fashioning out a variety with specific characteristics. The more diverse a genepool is, the higher is the probability that it would contain the desirable genes.

Secondly, there is a need to conserve genetic diversity. Change and development in agriculture and land use increase the risk that species, both cultivated and wild, will be lost in the future. It is a matter of moral principle to deter the extinction of useful plant species" (IUCN-UNEP-WWF, 1980). To quote Chang (1985), the preservation of genetic diversity is both a matter of insurance and investment for sustaining and improving future food resources." It, therefore, means that germplasm should be assembled not only to serve our current needs but they should also be conserved as a safeguard against unforeseen circumstances in the future.

Once the need to assemble a germplasm collection has been established, one can be assured that the collection will be a meaningful one and he can then proceed to determine the scope of the collection and the strategy to use in assembling it.

Actual exploration and collecting can be very expensive in terms of time, financial and personnel resources, therefore, effort should be allocated to planning a systematic and rational strategy to follow. Planning time may take from 3 to 12 months depending on the experience of the collecting team and their familiarity with the crop and the area.

Species Coverage and Species Prioritization

In setting priorities, the genepool concept of Harlan and de Wet (1971) which reflects ease of utilization can be used as a guide. In this concept, crop species and their relatives are grouped into the following:

Primary genepool (GP-1) - the true biological species including cultivated races and spontaneous races (wild and/or weedy). Within this genepool, crossing is easy. Hybrids among these are more or less fertile, chromosome pairing is good, segregation normal, and gene transfer to the crop is simple.

Secondary genepool (GP-2) - the coenospecies from whose members gene transfer is difficult but possible. Hybrids may be weak or partially sterile, chromosomes may pair poorly and there may be differences in ploidy level.

Tertiary genepool (GP-3) - from which moving genes is very difficult. Hybrids tend to be anomalous, lethal, or completely sterile. Gene transfer is either not possible with known techniques or may be possible but with the use of extreme radical measures such as the use of embryo culture, chromosome doubling or bridging species. This genepool represents the outermost limit for breeding by conventional means, but it is often ill defined due to lack of research.

The former International Board for Plant Genetic Resources (IBPGR) used four criteria in determining its crop priorities (IBPGR, 1981).

1. the risk that genetically diverse materials of the species and their wild relatives will be lost in the future, particularly the near future, as a result of change and development in agriculture and land use including the introduction of new varieties. Since such changes are generally local, a species or group may have high priority in one country or region and lower priority elsewhere;
2. the economic and social importance of the materials to be collected measured in terms of their present usefulness and importance (volume or value of production and trade, numbers of people depending on or using them), as well as their expected, intended or potential contributions to development (including the improvement of human diets and the income and well-being of farmers and other rural people) and the economic and social progress of mankind ;
3. the recognized requirements of plant breeders and research workers, in both developing and developed countries, for genetically diverse materials (including advanced breeding lines), and the expected significance in economic and social development of the improved types and varieties of crops they will be able to produce with these materials; and
4. the size, scope and quality, including documentation, of existing collections.

Based on the above criteria, the former IBPGR prepared a list of the priorities among crops in 1975, revised in 1981. Some countries have National Committees on Plant Genetic Resources which determine the priority species for collection and conservation. International Agricultural Research Centers with specific mandate crops and many smaller institutions have their own priority lists.

To further confirm the need for collecting, conduct a survey of the work that has been done. Prepare a listing of germplasm holdings for the species of interest. In this way one can make sure that there will be no useless duplication of effort. One must remember, as stated earlier, that a collection can also be assembled through donations and seed exchanges. A good reference on current germplasm

holdings is the IBPGR Directory of Germplasm Collections(IBPGR, 1990).

It is also necessary to evaluate the current status of the genetic resource of the species. Obtain the most up-to-date information on specific diversity of wild plants, threatened landraces, new varieties, hybrids and lines. If possible determine the total range of genetic variability within the species. Remember that a comprehensive collection carries the maximum diversity of the species. However, there are cases where the need to add to a collection is because there is recognized need for specific traits.

Access to germplasm holdings of other genebanks through the Internet has also made it easier to assess the present status of conservation for a particular crop species to avoid duplication and to pinpoint gaps.

Finally, decide on whether the mission will be a generalized one, covering many species e.g. all crop species, all wild species or a specialized collecting of a single crop. Where several crop species, related or unrelated grow together, multiple crop collecting would be cost effective and should be done. However, there are cases where each crop species occurs in distinct agroecological zones making multiple crop collecting impractical.

In the case of AVRDC, priority is on the principal crop species: bulb Alliums (onion, garlic, shallot), Chinese cabbage, common cabbage, eggplant, pepper, tomato, mungbean and soybean. Among the principal crops bulb Alliums and eggplant are among the least represented in the collection (Table 1). These are new crops of AVRDC and assembly of genetic diversity of these two crops was only started three years ago. Therefore, the Genetic Resources and Seed Unit (GRSU) of the Center is exerting effort to increase the size of the collection for these two crop species. Attempt was also made to evaluate representation of the genepool of the different crop species using the genepool concept of Harlan and de Wet (1971) (Figure 2). Effort was increased to fill the gaps in the collection especially in the primary and secondary genepool. Priority was also given to the assembly of specific traits e.g. descriptor states not fairly represented in the collection e.g. black-skinned eggplant, sources of resistance to pests and diseases. For the Center's principal crops, GRSU has also been very active in the acquisition of materials reported to possess desirable traits.

In the case of non-principal crops, AVRDC focuses on the conservation of landraces, farmer's selections, and heirloom varieties, genetic diversity that are in danger of being replaced by modern high yielding but highly uniform improved varieties. Prioritization is done in consultation with collaborators from the national programs.

Since vegetables include a wide range of species, it may also be helpful to group them into species that are highly domesticated, species that are gathered in the wild as well as encouraged and species that are not cultivated but only gathered in their wild state.

Geographic Coverage and Geographic Prioritization

The priority areas to be covered by the exploration mission can also be determined from the information used to determine species coverage and species prioritization. Divide the area into distinct regions based on either geobotanical data, political or administrative units.

The former IBPGR has also set criteria to determine priorities among regions (IBPGR. 1981)

1. that they contain significant genetic diversity of one or more crops or species selected accord-

ing to the criteria set for crops;

2. that change and development in agriculture, and/or change in land use, are occurring in them at such a rate that if nothing is done, genetically diverse materials are likely to be lost; and
3. that widespread crop failures have occurred or are imminent, or reasonably to be expected, on such a scale that diverse materials are likely, if nothing is done, to be lost.

Determine distribution and identification of the original habitats and areas occupied by the group(s). A visit to herbaria and a review of previous collecting trip reports in the area can provide these information. On a global scale, the concentration can be on the primary centers or origin or diversity, the secondary centers and sub-centers of diversity. Determine those parts in which the greatest intra-specific variability is to be found, and particularly the geographic centers in which endemic characters are most numerous. Determine similar patterns in cultivated and non-cultivated species. Survey past efforts. There may be no need to visit some areas.

Give due attention to ecological considerations Therefore, in planning the exploration and collecting mission consider also the following: clinal distribution vs mosaic distribution, parallel ecotypic differentiation and agroecological factors such as climate, topography, edaphic factors, altitude, cultivation pattern, and harvesting pattern.

Finally, based on the above, map out the existing collections and the areas proposed to be covered by the expedition. A good record of past collecting efforts or a distribution map of the origin of the present collection is a big help.

Time Considerations

There are physical limits to the time one can be out collecting and the amount of materials that can be collected. The duration of the collecting mission depends on favorable weather conditions and time of harvest of the species. It is therefore a good idea to have data on annual rainfall and distribution pattern as well as planting and harvest seasons for cultivated crops. The usual duration of a collecting expedition is 15 to 45 days. Sampling intensity varies from 10 to 20 samples per day. Therefore a collecting expedition usually can not expect to collect more than 1,000 samples.

If crops are grown in two different seasons it may be necessary to collect in both seasons since varieties grown may differ.

Implementing the Collecting Strategy

1. Assembly of the collecting team

The collecting team usually consists of three to five experts. If possible include one who knows the area of coverage and possesses a good command of the native language. Designate a team leader, who will coordinate all activities. Brief them on the significance of the germplasm collecting and conservation to be conducted. Also familiarize the team on the procedures to be followed and strategies to be taken during the collecting trip. It is best to include local counterparts in the collecting team.

The assembly of a collecting team can be done in conjunction with a mini-training session for germplasm collectors. This is very important since collecting may have to be done by local

counterparts throughout the year.

2. Collecting permits and quarantine requirements

Before embarking on the actual expedition, make sure that you have the necessary collecting permits. Most countries have definite policies on collecting of biological materials. Some countries (e.g. Philippines) require prior informed consent (PIC). Inform local authorities and observe order of protocol. A list of consulates and offices to contact would be helpful. Even within national boundaries there may be restrictions on movement of germplasm.

Inquire about quarantine regulations especially in the final destination of the collected materials. This will facilitate movement in the area to be explored and can avoid possible loss of expensively collected material. Collectors should have prop er inoculations and health certificates for international travel.

3. Materials needed for the expedition

Prepare a check list of equipment and supplies needed during the trip. One week before the scheduled collection trip prepare all the materials. Check off each item before setting out on the field trip (Table 2).

1.1 A fully covered motor vehicle, preferably a four wheel drive, which is rugged and versatile to reach remote areas is needed. The vehicle should always be in good condition during this kind of expedition trips. It should have enough room for the collecting team, the collecting supplies and the collected materials. The construction should be such that it would be easy for persons and materials to get in and out of. Information on road conditions can also help in deciding what type of vehicle to use.

1.2 Collecting forms, seed envelopes, cloth bags, net bags, tags, permanent writing pens, stapling wires and maps are the basic supplies needed for the trip.

1.3 Optional materials are chemicals for seed drying and seed treatment, and fixatives.

1.4 Official papers and letters to local agencies in the area and identification cards are necessary and could come in handy to avail help from local government officials.

4. Sampling procedures

If one is collecting a crop species that is sold in the market, a visit to the local market will give the collectors an idea of the diversity that may be expected and which ones are currently in the right stage of development. Interviews with the vendors may lead to the source of some materials.

The sampling procedure to be followed should aim at the fullest possible recovery of the genetic variation of the species, irrespective of the relative frequency or rarity of any genes or linked genetic complexes. The operational sampling unit is the field, the unit of ecotype distribution is the village or river basin or isolated valley. Sampling can also be done in the farmer(s seed store and the market place. However, experience has shown that very often seeds from these sources have low viability.

Always get the consent of the owner before sampling. Be very careful not to transgress cultural beliefs.

Random vs biased sampling. Field sampling is done by randomly selecting a starting point and taking seeds regularly as one goes through a series of transections within the fields so that proper sampling of the population is well represented in the collected samples. A biased sampling based on phenotypic selection is then added to the random samples. Biased sampling alone based on phenotypic selection is not recommended since phenotype is often modified by the environment. Sample only from healthy looking plants as much as possible at the time that seeds are at their highest viability.

Clustered sampling. For sampling wild species use a clustered pattern of sampling. The choice of this type of sampling is due to the difference in the population structures of wild species which are highly heterozygous as compared to traditional varieties.

Grids. Division of the area, even of the whole country into grids may be useful when the materials to be collected are almost evenly distributed throughout the area. It is also useful if prospecting is done and diversity analysis is used to decide on the samples to be taken.

Vegetables especially those in homegardens pose a problem in sampling. Very often a species is represented by only a few plants. In this case one has no choice but to sample from all the plants. Collect approximately the same amount of seeds from each plant.

5. Effective sample size

In field sampling, collect from as many plants as possible. For many crops, a random sample of 50 to 100 would be adequate (Marshall and Brown, 1975). If samples are not available in the field, farmers may be willing to share a limited amount of their stored materials.

For wild species, collect as many as possible representing the whole population since wild populations are heterogenous in nature.

Consider also the breeding structure of the species. As a guide one may follow the FAO-IPGRI recommendation for conservation. For homogeneous populations collect 1,500 to 2,000 seeds (IBPGR 1992) and more if there is reason to suspect that viability is less than 100%. For heterogeneous population collect more. Since regeneration is often a problem, think about the advantages and the possibility of collecting enough seeds for the base as well as the active collection.

6. Tags and labels

Tag and label samples; use water-proof permanent marking pens. Fill in duplicate tags with the following information. One label is to be put inside the container together with the collected material and the other is to be tied outside.

Collection No.:	LME-91-44
Collection Date:	3, Apr., 91'
Variety Name:	AZUCENA
Province:	LAGUNA

These information are also described in the collecting forms.

7. Sample containers

Put whole panicles/fruits in cloth bags or net bags, put one tag inside and close the bag by a string, then tie a duplicate tag on the bag. Net bags must be of a fine mesh so that small seeds (e.g. *Amaranthus, Brassica*) will not pass through. Avoid the use of plastic bags since moisture and heat can accumulate inside and may cause loss of viability. For unthreshed samples (e.g. *Amaranthus, Brassica,* legumes) the conditions inside the plastic bag is favorable to growth of molds.

For seed samples, put threshed samples in paper envelopes. label the envelopes, put one tag inside and close using a stapler.

A big nylon net bag may be used for large fruits (e.g. squash, cucumber, bottle gourd, etc.)

8. Processing of samples

Process samples at the collection site especially if they are wet and bulky for transport. For succulent fruits (e.g. tomato, *Coccinia,* etc.) process immediately using wet extraction. Wash the seeds very well and dry. Preliminary drying is important to prevent the growth of molds. Drying should be done only by aeration. Do not sun dry or put on hot concrete floors. This may affect the viability of the seeds. An air-conditioned room is good for the initial drying.

If threshing is done, put the seeds of a sample in a paper envelope, label properly and transfer the tag inside the envelope.

Remember though that processing at the collecting site is not enough. The seeds must be processed further at their destination before they go into final storage.

9. Sample packing for transport

Place properly processed seeds in paper envelopes and send directly to their destination. The collected materials must reach the destination genebank in the shortest time possible. Send samples by courier, the soonest after collecting the samples. Do not wait until you have accumulated a large number of samples. This delay may affect the viability of the seeds if not processed immediately.

When transporting germplasm to different areas always follow local or international quarantine measures implemented in the area.

10. Documentation procedures during collecting

Collecting data, if properly taken can provide many useful information e.g. adaptive characters, genetic and breeding potential, ecological properties, etc.

A collecting form has been designed by the International Board for Plant Genetic Resources. This form is very similar to the ones used by the International Rice Research Institute and the Asian Vegetable Research and Development Center. Although these forms take some time to fill out and may be cumbersome, they must nevertheless be filled out in the collecting site. Very often it is after one has finished with the collecting mission that one realizes how important filling out the forms is.

Fill-up collecting forms as completely as possible during or right after the collection of the

samples.

Photograph the area or sampling sites or anything that can provide information about the sample and the collecting site. This could be very useful in the presentation of the trip report and the documentation process of the field collecting report. A caution about taking photographs. Be sure that photographing the area is allowed. Keep in mind that there are also international and some national laws on documenting indigenous knowledge e.g. photographing and recording may not be allowed in some cases.

A field collecting notebook filled up like a diary will be useful in the preparation of the field collecting report.

Interviews

Always interview the farmer owner of the collected germplasm, vendors or any other person who can provide information related to the materials being collected. Include questions that will reveal indigenous knowledge about biodiversity and its conservation, usage and even folklore as well as linguistic information associated with the material being collected. Include also information on the role of family members in the conservation of diversity.

Field Collecting Report

The team leader should prepare a field collecting report that is as informative as possible. It should cover the itinerary, highlights of observations on local flora, cultural practices and annotated maps as well as photographs showing collecting sites ecological features and other information. The collecting report is best done as soon as the collector reaches his base station while memories of the details of the exploration are still fresh in his mind. If the collector has a well kept journal regularly filled during collecting, the report will be easy to prepare.

The field collecting report and the collecting forms are official documents that properly acknowledge the collector and his agency as the ones to be credited for the collecting activity. It can later be published so that any paper or report that makes use of the collection can site the report.

Distribution of the Collected Material and Information

There should be a scheme for depositing materials in the home country, international research centers and the center of the proponent. The plant materials should be accompanied by at least the passport data and the field collecting report.

Conclusion

A well planned collecting expedition will result in a good collection representing the maximum diversity in the species and information that would be useful not only in the utilization of the collected material but in understanding the ecology and other biological properties of the species. It will also avoid unnecessary expense in time, financial resources and personal services.

The assembly of individuals that represent the genepool of the species is only the initial step in genetic conservation. Since it entails considerable time, effort and money there should be serious at-

tempt to preserve and utilize the information and materials generated from the exercise.

References

Bakhareva, S.N. 1990. The USSR policy for exchanging genetic resources and the germplasm collection procedures of the Vavilov Institute. Diversity 6 (3&4):10-12.

Brown, A.H.D., O.H. Frankel, D.R. Marshall and J.T. Williams (Eds.). 1989. The Use of Plant Genetic Resources. Cambridge University Press, Cambridge, New York, New Rochelle, Melbourne or Sydney. 382 pp.

Chang, T.T. 1985. Principles of genetic conservation. Iowa State Journal of Research 59: 325-348.

Chang, T.T. 1985. Collection of crop germplasm. Iowa State Journal of Research 59:349-364.

Clements, R. J. and D. G. Cameron (Eds.) 1980. Collecting and Testing Tropical Forage Plants. Commonwealth Scientific and Industrial Research Organization. Melbourne, Australia. 154 pp.

Frankel and Bennet (Ed.). 1970. Genetic Resources in Plants - Their Exploration and Conservation. IBP Handbook No. 11. F.A. Davis Co., Philadelphia. 554 pp.

Frankel, O.H. and J.G. Hawkes (Ed.). 1975. Crop Genetic Resources for Today and Tomorrow. Cambridge University Press, Cambridge, London, New York or Melbourne. 492 pp.

Guarino, L., V. Ramanatha Rao, and R. Reid (Eds.). 1995. Collecting Plant Genetic Diversity. Technical Guidelines. CAB International.

Harlan, J.R. 1956. Distribution and utilization of natural variability in cultivated plants. Brookhaven Symposia in Biology 9:191-208.

Harlan, J.R. and J.M.J de Wet. 1971. Toward a rational classification of cultivated plants. Taxon 20: 509-517.

Hawkes, J.G. 1976. Manual for Field Collectors (seed crops). AGPE, Misc/7. FAO, Rome.

Hawkes, J.G. 1980. Crop Genetic Resources Field Collection Manual. International Board for Plant Genetic Resources and European Association for Research on Plant Breeding (EUCARPIA). Druk Pudoc, Wageningen. 37 pp.

IBPGR. 1981. Revised Priorities Among Crops and Regions.

IBPGR. 1990. Directory of Crop Germplasm Collections. 4. Vegetables: *Abelmoschus, Allium, Amaranthus,* Brassicaceae, *Capsicum,* Cucurbitaceae, *Lycopersicon, Solanum* and other vegetables. Bettencourt, E. and Konopka, J. International Board for Plant Genetic Resources, Rome. 250 pp.

IBPGR. 1992. International Board for Plant Genetic Resources. Annual Report 1992.

Marshall, D. R. and A. H. D. Brown. 1975. Optimum sampling strategies in genetic conservation. In Frankel, O. H. and J. G. Hawkes (Ed.). 1975. Crop Genetic Resources for Today and Tomorrow. Cambridge University Press, Cambridge, London, New York or Melbourne. pp. 53-80

Vavilov, N. I. 1951. The origin, variation, immunity and breeding of cultivated plants. Chron. Bot. 13:1-364. Cited in Frankel and Bennet (Ed.). 1970. Genetic Resources in Plants - Their Exploration and Conservation. IBP Handbook No. 11. F.A. Davis Co., Philadelphia. pp. 554.

Table 1. Vegetable germplasm maintained at AVRDC, as of 31 October 1997.

Crop	TOTAL
Principal crops:	
Soybean	14, 063
Tomato	7, 146
Pepper	6, 861
Mungbean	5, 785
Eggplant	2, 253
Brassica	1, 522
Allium	1, 030
Subtotal	38, 660
Non-principal crops:	
Yardlong bean and cowpea	1, 628
Phaseolus bean (including lima bean)	566
Black gram	452
Luffa	286
Cucumis	275
Abelmoschus	230
Pea	211
Cucurbita	198
Lablab bean	175
Benincasa	133
Others	1, 319
TOTAL	44, 133

Table 2. Checklist of materials needed for a collecting expedition.

Vehicle
Collecting forms
Journal for field notes
Camera and film
Checklist of plants to be collected: botanical name, synonyms, folknames, distribution uses and plant parts used.
A good flora of the region
Paper, cloth , net bags, plastic bags
Tags and labels
Rubber bands
Masking tapes
Cords
Scissors
Pruning shears
Permanent marking pens
Pencils
Spade
Bolo
Altimeter
Magnifying glass
measuring tape
Herbarium press
Gloves
Boots
First aid kit

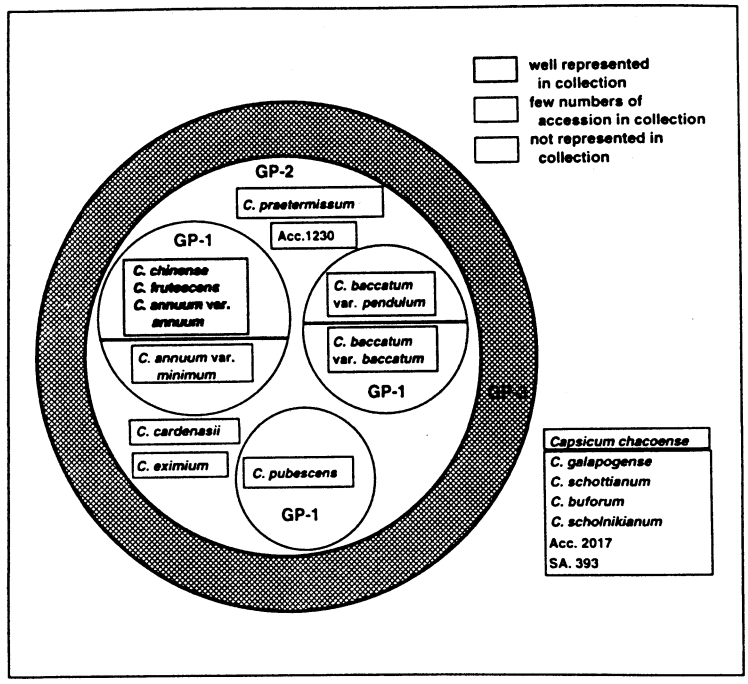

Figure 1. Representation of the Capsicum genepool in the pepper germplasm collection at AVRDC.